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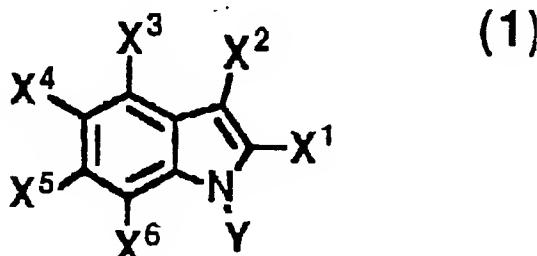
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(54) Agents for controlling underwater fouling organisms.

(57) The present invention provides the use as undersea anti-fouling reagent of an indole compound of Formula (1) :



(wherein Y represents hydrogen, lower alkyl, or phenyl ; X<sup>1</sup>, X<sup>2</sup>, X<sup>3</sup>, X<sup>4</sup>, X<sup>5</sup> and X<sup>6</sup> independently represent hydrogen, halogen, lower alkyl, lower alkoxy, nitro, cyano, cyano lower alkyl, halogenated lower alkyl, di-lower-alkylamino-lower alkyl, etc.) The compounds of formula (1) are safe and effective underwater anti-fouling reagents.

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1. Introduction

The present invention relates to agents that prevent unwanted fouling organisms from attaching and growing on ship hulls, culturing nets, set-nets, sea equipments such as buoys, the cooling water tubes of atomic power plants or thermal electric power plants, and the inlet channels of heat exchangers in the petrochemical industry.

2. Background of the Invention

In ship hulls, underwater structures, the inlet channels of a cooling water tubes and other structures that are always exposed to water, there are various shellfish and algae such as *Balanus*, *Mytilus*, *Hydrozoa*, *Ulva*, *Enteromorpha* as well as others that attach and grow, or even overgrow. The attachment of these organisms to these structures causes economic damage in various forms: increased attachment to ship hulls slows down the cruising speed of these vessels, increases fuel consumption and also causes serious economical loss due to the cancellation of service in order to clean the ship hulls. Their attachment to cooling water equipment decreases heat conductivity which eventually reduces the cooling power of the equipment.

A variety of agents useful for controlling fouling organisms in fresh water or sea water have been used to prevent the attachment and overgrowth of these organisms. Copper compounds and organotin compounds are known active anti-fouling agents, and are still extensively used today. Anti-fouling agents in the form of a paint can contain 10-50% by weight of the active ingredient and can be used to paint surfaces such as ship hulls. The paint prevents attachment and growth of fouling organisms by continuously releasing anti-fouling agents underwater.

3. Summary of the Invention

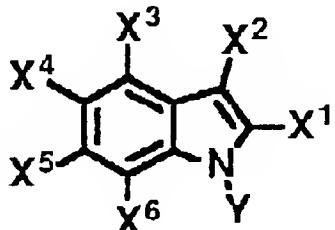
The reagents of the present invention have excellent anti-fouling properties against underwater fouling organisms. The reagents are more effective than conventional anti-fouling agents containing heavy metals and more importantly, are safe for our health and the environment.

4. Detailed Description of the Invention

Environmental problems such as polluted rivers and sea with heavy metals and toxic compounds have been caused by the industrial use of such materials. For example, stern warnings have been issued regarding the effects of heavy metal compounds, especially organotin compounds which are widely used and known to be effective anti-fouling reagents because of their effect on the natural environment and on our health when accumulated in our body via food such as fish and shellfish. Therefore, there is a need for the development of safe and effective anti-fouling compounds.

The present inventors have attempted to develop highly-safe, and effective anti-fouling compounds. It has been found that particular indole compounds have an excellent repellent effect against fouling organisms. The present invention provides anti-fouling reagents comprising, as active ingredients, indole compounds represented by Formula(1):

(1)



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(wherein Y represents hydrogen, lower alkyl, or phenyl; X<sup>1</sup>, X<sup>2</sup>, X<sup>3</sup>, X<sup>4</sup>, X<sup>5</sup> and X<sup>6</sup> independently represent hydrogen, halogen, lower alkyl, lower alkoxy, nitro, cyano, cyano lower alkyl, halogenated lower alkyl, substituted or unsubstituted phenyl, aralkyl, aralkyloxy, lower alkoxy carbonyl, aliphatic lower acyl, aliphatic lower acyloxy, lower-alkoxycarbonyl-lower alkyl, aromatic acyl, lower-cycloalkylamino-lower alkyl, nitrovinyl, pyridyl lower alkyl, carbamoyl lower alkyl, carbamoyl, diallyl amino lower alkyl or di-lower-alkylamino-lower alkyl).

In the formula (1), lower alkyl represented by X<sup>1</sup>, X<sup>2</sup>, X<sup>3</sup>, X<sup>4</sup>, X<sup>5</sup>, X<sup>6</sup> or Y refers to a straight or branched

alkyl group having 1-5 carbon atoms and includes methyl, ethyl, propyl, isopropyl, sec-butyl, tert-butyl, pentyl and hexyl. Examples of halogen represented by X<sup>1</sup>, X<sup>2</sup>, X<sup>3</sup>, X<sup>4</sup>, X<sup>5</sup> or X<sup>6</sup> includes a fluorine, chlorine, bromine or iodine atom. Lower alkoxy refers to an alkoxy group having 1-5 carbon atoms and includes methoxy, ethoxy, propoxy and butoxy. Cyano lower alkyl refers to said lower alkyl substituted by a cyano group. Examples of 5 cyano lower alkyl includes a cyanomethyl, cyanoethyl and cyanopropyl group. Halogenated lower alkyl refers to said lower alkyl substituted by said halogen. Examples of halogenated lower alkyl includes a chloromethyl, trifluoromethyl and 2-bromoethyl group. Examples of substituted phenyl includes a phenyl group substituted by amino group, said halogen, said lower alkyl or said lower alkoxy. Examples of aralkyl include a benzyl group. Examples of aralkyloxy include a benzyloxy group. Lower alkoxy carbonyl refers to carbonyls having 2-6 carbon 10 atoms and includes methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, butoxycarbonyl. Aliphatic lower acyl refers to compounds having 2-6 carbon atoms and includes acetoxy and propionyloxy. Lower-alkoxycarbonyl-lower alkyl refers to said lower alkyl substituted by said lower alkoxy carbonyl and lower-alkoxycarbonyl-lower alkyl includes methoxycarbonylmethyl and ethoxycarbonylethyl. Examples of an aromatic acyl include unsubstituted benzoyl or a benzoyl group substituted by amino, said halogen, said lower alkyl, or said lower alkoxy; 15 Lower-cycloalkylamino-lower alkyl means said lower alkyl substituted by lower cycloalkylamino having 3-7 carbon atoms. Examples of lower-cycloalkylamino-lower alkyl include cyclohexylaminomethyl, cyclohexylaminoethyl, cyclopentylaminomethyl and cyclopentylaminoethyl. Pyridyl lower alkyl refers to said lower alkyl substituted by pyridyl and examples of pyridyl lower alkyl includes pyridylmethyl, pyridylethyl, and pyridylpropyl, Carbamoyl lower alkyl refers to said lower alkyl substituted by carbamoyl and examples of carbamoyl lower alkyl 20 includes carbamoylmethyl, carbamoylethyl and carbamoylpropyl. Diallylamino-lower alkyl refers to said lower alkyl includes diallylaminomethyl, diallylamoethoxyethyl and diallylaminopropyl. Di-lower-alkylamino-lower alkyl means said lower alkyl substituted by di-lower-alkylamino and examples of di-lower-alkylamino-lower alkyl includes dimethylaminomethyl, dimethylaminoethyl and dimethylaminopropyl.

Examples of indole compounds represented by the Formula(1) include but not limited to those listed in 25 Table 1.

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Table 1

5	Compound No.	Chemical name
10	1	2,3-Dimethylindole
	2	3-Indolylacetonitrile
	3	3-Indolylacetate
	4	Ethyl 5-chloro-2-indolecarboxylate
15	5	1-Methylindole
	6	5-Methylindole
	7	3-(2-Nitrovinyl)-1-phenylindole
	8	4-Chloroindole
20	9	6-Chloroindole
	10	5,6-Dimethoxyindole
	11	4-Methoxyindole
25	12	4-Methylindole
	13	6-Methylindole
	14	4-Indolylacetate
	15	4-Nitroindole
30	16	Methyl 4-indolecarboxylate
	17	Ethyl 3-indoleacetate
	18	3-Indolylacetamide
	19	4-Methoxy-1-methylindole
35	20	3-Cyanoindole
	21	1,2-Diphenylindole
	22	Methyl 4-methoxy-2-indolecarboxylate
40	23	Methyl 6-methoxy-2-indolecarboxylate
	24	Methyl 4,6-dimethoxy-2-indolecarboxylate
	25	Dimethyl 2,3-indolecarboxylate
	26	3-(2-Bromoethyl)indole
45	27	3-Acetylindole
	28	5-Benzylxyindole
	29	5-Bromoindole
	30	5-Chloroindole
50	31	5-Chloro-2-methylindole
	32	5-Cyanoindole

	33	1,2-Dimethylindole
	34	2,5-Dimethylindole
5	35	Ethyl 2-indolecarboxylate
	36	5-Fluoroindole
	37	5-Methoxyindole
10	38	5-Methoxy-2-methylindole
	39	2-Methylindole
	40	3-Methylindole
	41	7-Methylindole
15	42	5-Nitroindole
	43	6-Nitroindole
	44	2-Phenylindole
20	45	5-Bromo-3-indolylacetate
	46	3-Carbamoyl-2-methylindole
	47	1-Ethyl-2-phenylindole
	48	1-Methyl-2-phenylindole
25	49	1-Methyl-3-indolylacetate
	50	3-(Cyclohexylaminomethyl)indole
	51	2-Methyl-3-propionylindole
30	52	5-Benzylxy-3-indolylacetamide
	53	5-Benzylxy-3-indolylacetonitrile
	54	Ethyl 5-benzylxy-2-indolecarboxylate
	55	Ethyl 5-methyl-2-indolecarboxylate
35	56	2-Methyl-3-propylindole
	57	3-Benzylindole
	58	Methyl 3-(3-indolyl)propionate
	59	3-Propionylindole
40	60	3-(3-Indolyl)propionitrile
	61	2,3-Dimethyl-5-nitroindole
	62	3-Diallylaminomethylindole
45	63	Methyl 3-indolylacetate
	64	3-(2-Nitrovinyl)indole
	65	3-[2-(3-Pyridyl)ethyl]indole
	66	3-Ethyl-2-methyl-5-nitroindole
50	67	5-Chloro-2-phenylindole
	68	3-(4-Chlorobenzoyl)-2-methylindole
	69	2-(2-Aminophenyl)indole

	70	Gramine
5	71	1-Methylgramine
	72	2-Methylgramine
	73	5-Methylgramine
	74	6-Methylgramine
10	75	5-Methoxygramine
	76	5-Fluorogramine
	77	6-Fluorogramine
15	78	5-Bromogramine
	79	6-Bromogramine
	80	4-Chlorogramine
	81	5-Chlorogramine
20	82	5-Benzylxygramine
	83	6-Benzylxygramine
	84	1,2-Dimethylgramine
	85	2,6-Dibromogramine
25	86	5,6-Dibromogramine
	87	6-Bromo-2-methylgramine
	88	2,5,6-tribromogramine
30	89	5,6-Dibromo-2-methylgramine
	90	2,6-Dibromo-1-methylgramine
	91	2,5,6-Tribromo-1-methylgramine
	92	N,N-dimethyl-5-methoxytryptamine
35	93	N,N-dimethylhomotryptamine

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40 The indole compounds shown in Table 1 are all known compounds and can be chemically synthesized by conventional methods known in the art.

Most of the indole compounds described above are found in animals, plants, and bacteria and can be obtained by extraction and purification from these organisms. For example, 2,5,6-Tribromo-1-methylgramine (Compound No.91), an indole compound represented by the formula(1) wherein X<sup>1</sup>,X<sup>4</sup> and X<sup>5</sup> are bromine; X<sup>3</sup> and X<sup>6</sup> are hydrogen; X<sup>2</sup> is dimethylaminomethyl; and Y is methyl, is known to be found in *Zoobotryon verticillatum*, a species of Bryozoa [Aiya Sato et al., Tetrahedron Lett., 24, 481. (1983)]. In addition, Compound No.91 can also be extracted and purified from *Zoobotryon pellucidum*, as is disclosed by the present inventors.

Solvents used for extracting these compounds include typical organic solvents, preferably acetone or ethanol. Purification methods include those known in the art such as partition chromatography, preferably silica gel chromatography using a mixture of hexane and ethyl acetate as an eluent.

The indole compounds used as the active ingredient in the present invention may be used alone or in combination with other anti-fouling agents. Active ingredients extracted from organisms may be used as a crude extract or purified, if necessary. The anti-fouling compounds of the present invention may be used by formulating the agents in numerous forms including as a paint, a solution and as an emulsion. Formulation is carried out as usual without any difficulties.

For example, when the reagents of the present invention are used as a paint, an anti-fouling paint is prepared by formulating one of the active ingredients described above with other components as described below. The anti-fouling paint can then be applied to ship hulls, underwater structures and inlet channels of water cool-

ong systems. The anti-fouling paint comprises the active ingredient of the present invention and film-forming ingredients, including solvents which are selected according to use, extender pigments, coloring pigments and additives. Film-forming ingredients includes chlorinated rubber resin, vinyl acetate resin, acrylic resin and natural resin. The active ingredient is typically formulated 1-45% by weight based on the weight of the anti-fouling point, preferably 5-20%.

When the reagents of the present invention are used as a solution, an anti-fouling solution is prepared by formulating the active ingredients with film-forming ingredients as described above and by dissolving the mixture in solvents.

The anti-fouling solution is then applied to farming nets and set-nets in order to prevent overgrowth of the 10 fouling organisms. Film-forming ingredients used in the anti-fouling solution include chlorinated rubber resin, vinyl acetate resin, acrylic resin and natural resin. Solvents include toluene, xylene, cumene, methylisobutylketon, ethyl acetate and methanol. Additives such as a plasticizer can be added to the anti-fouling solution, if necessary. The active ingredient is typically formulated 2-70% by weight based on the weight of an anti-fouling solution, preferably 5-30%.

15 When the agent is employed as an emulsion, an anti-fouling solution is prepared according to the conventional method in the art, by dissolving active ingredients described above in solvents and by adding surfactants to the mixture. Surfactants include those typically used in the art. When the agents of the present invention are used as emulsion, the active ingredient is typically formulated 5-50% by weight based on the weight of anti-fouling emulsion, preferably 10-40%. The anti-fouling reagents of the present invention may be used by 20 kneading the agents into polymer resin materials of fishing nets and the materials of underwater structure such as concrete.

## 5. Examples

25 The present invention will be more readily understood with reference to the following examples. However, these examples are intended to illustrate the present invention and are not to be construed to limit the scope of the invention.

### Example 1 Effect of indole compounds in Table 1' on *Balanus amphitrite* and *Artemia*

30 Various indole compounds were tested for their affect on attachment of the larvae of *Balanus amphitrite*, an infamous fouling organism, as well as their affect on other beneficial organisms such as *Artemia salina*, a zooplankton used as feed for juvenile fish.

35 Selected compounds in Table 1, 0.05mg each, were dissolved in 0.1ml methanol. Round plates (4cm in diameter) were then uniformly coated with the solution and air-dried to evaporate methanol. 5ml of filtrated sea water, 10 larvae of *B. amphitrite* in the attachment stage of the development, and 5 larvae of *A. salina* were placed in the each plate. The plate was incubated in the darkroom at 23 °C for 24 hours. After 24-hour incubation, the number of molted *B. amphitrite* attached to the bottom of the plate was counted to evaluate the effect of the test compound and the number of dead *A. salina* was also counted to evaluate the safety of 40 the compound for beneficial organism.

As comparison, a similar test was carried out using 0.05 mg of Bis(tributyltin)oxide (hereafter referred to as "TBTO") instead of the compound in Table 1.

The above test was repeated three times and the resulting numbers were averaged. Table 2 shows the results. The compound number in Table 2 corresponds the one in Table 1.

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Table 2

	Compound No.	% Attachment rate of larvae Mortality of <i>Artemia</i>	
		Attachment rate of larvae	Mortality of <i>Artemia</i>
50	2	0	0
	4	0	0
55	6	0	0
	8	0	0
	10	5	0

	12	5	0
5	14	10	0
	16	10	0
	18	15	0
	20	0	0
10	22	0	0
	24	0	0
	26	0	0
	28	0	0
15	30	0	0
	32	0	0
	34	0	0
20	36	0	0
	38	0	0
	40	0	0
25	42	0	0
	44	5	0
	46	10	0
30	48	10	0
	50	0	0
	52	15	0
	54	0	0
35	56	0	0
	58	0	0
	60	0	0
40	62	15	0
	64	0	0
	66	0	0
45	68	10	0
	TBTO	0	100
	non-agent	85	0

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Example 2 Comparison of the anti-fouling reagents of the invention with Copper Sulfate and TBTO

Various indole compounds of formula(1) wherein X<sup>2</sup> is the di-lower-alkylamino-lower alkyl, were tested for effect on the attachment of larvae of *B. amphitrite*.

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Selected compounds in Table 1, 0.05 mg and 0.005 mg each, were dissolved in 0.1 ml methanol. Round plates (4 cm in diameter) were then uniformly coated with the solution and air-dried for removing methanol. 5 ml of filtrated sea water, 10 larvae of *B. amphitrite* in the attachment stage of the development were placed in each plate. The plate was incubated in the darkroom at 23 °C for 24 hours. After 24-hour incubation, the number

of molted *B. amphitrite* attached to the bottom of the plate was counted to evaluated the effect of the test compound.

As a comparison, a similar test was carried out using Copper sulfate and TBTO instead of the compound in Table 1. The above test was repeated three times and the resulting numbers were averaged, Table 3 shows the results. The compound number in Table 3 corresponds the number used of Table 1.

Table 3

10	Compound No.	Dosage(mg/5ml) and attachment rate of larvae (%)		Condition of non- attached larvae
		0.05mg	0.005mg	
15		0.05mg-dosage		
	70	0	35	vigorously swim
	71	0	15	vigorously swim
20	72	0	0	vigorously swim
	73	0	5	vigorously swim
	74	0	5	vigorously swim
25	75	0	0	vigorously swim
	76	0	0	vigorously swim
	77	0	0	vigorously swim
	78	0	0	vigorously swim
30	79	0	0	vigorously swim
	80	0	5	vigorously swim
	81	0	0	vigorously swim

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	82	0	0	vigorously swim
5	83	0	0	vigorously swim
	84	0	10	vigorously swim
	85	0	0	vigorously swim
	86	0	0	vigorously swim
10	87	0	0	vigorously swim
	88	0	0	vigorously swim
	89	0	0	vigorously swim
	90	0	0	vigorously swim
15	92	0	5	vigorously swim
	93	0	35	vigorously swim
	Copper Sulfate	0	30	all larvae died
20	TBTO	0	0	all larvae died
	Non-agent	85		

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As is evident from Table 3, Copper Sulfate and TBTO prevent *Balanus* from attachment based on their toxicities, otherwise, the reagents of the present invention prevent the organisms from attaching without killing them.

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#### Example 3 Compound No.91 obtained from *Zoobotryon pellucidum*

2.1Kg of *Zoobotryon pellucidum* (phylum Tentaculata, class Bryozoan) were extracted with 3 Kg of acetone. After extraction, acetone was removed under reduced pressure and the residue was partitioned with 800 ml ethyl acetate and 1200 ml water. The ethyl acetate layer was separated and evaporated under reduced pressure. The resulting residue was purified by silica gel chromatography using an eluent of 30 % ethyl acetate in hexane (v/v) to give 610 mg of Compound No.91.

In the analyses, <sup>1</sup>H-NMR spectrum, visible light absorption spectrometry, and mass spectrometry, the compound thus obtained was found to be equivalent to the compound in the art.

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#### Example 4 Comparison of the anti-fouling effect of Compound No.91 with Copper Sulfate and TBTO

Compound No.91 obtained in Example 3 was tested for effect on attachment using the larvae of *B. amphitrite*.

45 0.05 mg of Compound No.91 was dissolved in 0.1 ml methanol. Round plates (4 cm in diameter) were then uniformly coated with the solution and air-dried to remove methanol. 5 ml of filtrated sea water, 10 larvae of *B. amphitrite* in the attachment stage of the development were placed in each plate. The plate was incubated in the darkroom at 23 °C for 24 hours. After 24-hour incubation, the number of molted *B. amphitrite* attached to the bottom of the plate was counted to evaluate the effect of the test compound. As a comparison, a similar 50 test was carried out using 0.1 mg of Copper Sulfate, 0.05 mg of TBTO and the absence of any agent instead of Compound No.91. Table 4 shows the results.

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Table 4

Test plot	Active ingredient	Dosage (mg)	Attachment rate (%)	Condition of non-attached larvae
Example 4	Compound No.91	0.05	0	Vigorously swim (50%)
Comparison 4	Copper Sulfate	0.1	0	All larvae died
Comparison 5	TBTO	0.05	0	All larvae died
Comparison 6	Non-agent	0	70	Vigorously swim (100%)

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**Example 5 Formulation of the anti-fouling reagents of the invention**

When the anti-fouling reagents of the present invention is used as a preventive paint for the attachment of fouling organisms, the formulation is as follows.

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Formulation 1

Composition	Weight (%)
Invention compound	15
Rosin WW	6
VYHH(synthetic vinyl resin)	6
Tricresyl phosphate	2
Talc	15
Barium sulfate	15
Red iron oxide	10
Xylene	26
Methylisobutylketon	5
Total	100

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## Formulation 2

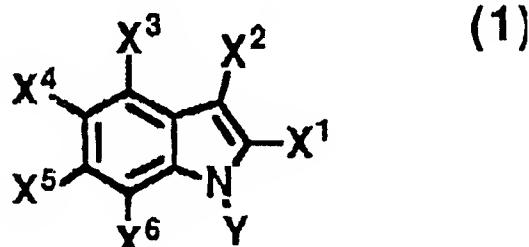
	Composition	Weight (%)
5	Invention compound	10
	Rosin WW	6
	VYHH(synthetic vinyl resin)	6
10	Tricresyl phosphate	2
	Talc	20
	Barium sulfate	15
15	Red iron oxide	10
	Xylene	20
	Methylisobutylketon	11
20	Total	100

## Claims

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1. The use as underwater anti-fouling reagent of an indole compound of Formula (1):

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(wherein Y represents hydrogen, C<sub>1</sub>-C<sub>5</sub> alkyl, or phenyl; X<sup>1</sup>, X<sup>2</sup>, X<sup>3</sup>, X<sup>4</sup>, X<sup>5</sup> and X<sup>6</sup> independently represent hydrogen, halogen, C<sub>1</sub>-C<sub>5</sub> alkyl, C<sub>1</sub>-C<sub>5</sub> alkoxy, nitro, cyano, cyano C<sub>1</sub>-C<sub>5</sub> alkyl, halogenated C<sub>1</sub>-C<sub>5</sub> alkyl, substituted or unsubstituted phenyl, aralkyl, aralkyloxy, C<sub>2</sub>-C<sub>6</sub> alkoxy carbonyl, aliphatic C<sub>2</sub>-C<sub>6</sub> acyl, aliphatic C<sub>2</sub>-C<sub>6</sub> acyloxy, C<sub>2</sub>-C<sub>6</sub> alkoxy carbonyl- C<sub>1</sub>-C<sub>5</sub> alkyl, aromatic acyl, C<sub>3</sub>-C<sub>7</sub> cycloalkylamino- C<sub>1</sub>-C<sub>5</sub> alkyl, nitrovinyl, pyridyl C<sub>1</sub>-C<sub>5</sub> alkyl, carbamoyl C<sub>1</sub>-C<sub>5</sub> alkyl, carbamoyl, diallylamino C<sub>1</sub>-C<sub>5</sub> alkyl or di- C<sub>1</sub>-C<sub>5</sub> alkylamino -C<sub>1</sub>-C<sub>5</sub> alkyl).

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2. An underwater anti-fouling reagent composition comprising as active ingredient an indole compound of Formula (1) as defined in claim 1.

3. A composition according to claim 2 formulated as a paint, solution or emulsion.

4. A method of producing an indole compound of Formula (1) wherein X<sup>1</sup>, X<sup>4</sup> and X<sup>5</sup> are bromine, X<sup>3</sup> and X<sup>6</sup> are hydrogen, X<sup>2</sup> is dimethylaminomethyl and Y is methyl, which method comprises extracting said compound from Zoobotryon pellucidum and purifying said compound.

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